Comparison of Umbilical Cord Interleukin-8 in Low Birth Weight Infants with Premature Rupture of Membranes and Intact Membranes

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Abstract: Some studies showed increased levels of proinflammatory cytokines like IL-6, IL-8 and TNF-α in the blood samples of pregnant women with PROM (Premature rupture of membranes) and their neonates. The aim of this study was to find a relationship between increased level of IL-8 and PROM, a cost benefit method for early diagnosis and reduction of hospitalization period of neonatal sepsis. This case control study was conducted in Obstetrics and Gynecology Department of Al-Zahra Hospital at Tabriz University of Medical Sciences, Iran from 10th April 2001 to 20th June 2003. We studied 50 LBW (Low birth weight) neonates born from mothers with PROM as the case group and fifty LBW neonates born from mothers without PROM as our control group. Neonates born from pregnant women with PROM underwent sepsis workup and blood samples from their umbilical cord were sent for blood culture and IL-8 level measurement. Mean levels of IL-8 in study and control groups were 128.12 and 39.2 pg mL⁻¹, respectively. We had no positive blood culture and no bacteria could be isolated. Significantly elevated values (p<0.0003) were showed in cases with PROM compared to cases without PROM (medians 67.5 pg mL⁻¹ vs. 29.5 pg mL⁻¹, respectively). This study showed a strong relationship between IL-8 elevation and PROM. Increased levels of IL-8 can be used as indicator for early diagnosis of neonatal sepsis.

Key words: Premature rupture of membranes, IL-8, neonatal sepsis

INTRODUCTION

Premature Rupture of Membrane (PROM) is spontaneous rupture of fetal membrane before the onset of labor at any gestational age. The incidence of PROM in all pregnancies is approximately 8%, most occurring at term (Romero and Mazor, 1988). The amniotic fluid surrounding the developing fetus is important for the developing of fetal lungs, limbs movements, heat exchange and protection of umbilical cord and fetus from compression. If the fetal membranes rupture these protective mechanisms may compromised, if the fluid is lost then maternal and fetal complication arise (Shobokshi and Shaaawy, 2002). Birth before 37 weeks of gestation is a major cause of fetal and neonatal morbidity and mortality. Problems due to prematurity or low birth weight have been well documented and these factors account for 60% of all neonatal mortality (Shapiro et al., 1988). Gibbs and colleagues reported that premature rupture of the fetal membranes (PROM) is associated with one third of all preterm deliveries and is the third leading causes of perinatal death (Gibbs and Blanco, 1982).

The etiology of preterm birth and PROM is multifactor and an exact cause in each case can rarely be identified. Recent evidence suggests a strong association between infection, preterm labor (PTL) and PROM. Intra-Amniotic Infection (IAI) and the host inflammatory response can lead to the accumulation of proinflammatory cytokines, many of which are known to induce the release of uterotonin substances that cause the uterus to contract like prostaglandin from gestational tissues, thereby causing uterine contractions and labor (Romero and Mazor, 1988; Fortunato et al., 2002). There is a widespread hypothesis that lower genital tract infections have been associated with increased Premature Rupture of Membrane (PROM). The role of bacteria in PROM is under investigation and there are several mechanisms by which bacteria could increase the incidence of PROM. Some authors believe that bacteria activate host defense macrophage system and it results in preferential activity leading to the secretion of uterotonin agents such as prostaglandins (PG), interleukins (IL), etc. and then PROM may result (Yoon et al., 1995; Stoll, 2001). In this case-control study we measured IL-8 levels in blood samples
taken from umbilical cords of neonates born from mothers with PROM to find out the association between cytokines and PROM.

MATERIALS AND METHODS

The study was carried out in Obstetrics and Gynecology Department of Al-Zahra Hospital, a tertiary and teaching hospital attached to Tabriz University of Medical Sciences. The primary purpose was to identify the relationship between IL-8 levels in umbilical cord blood of low birth weight neonates with preterm rupture of membranes and comparing them with their blood culture. Study cases were selected from women who were admitted to the Obstetrics ward between 10/04/2001 to 20/06/2003. We studied fifty LBW (birth weight less than 2500 g) neonates born from mothers with PROM as case group and fifty LBW neonates born from mothers without PROM as control group. Neonates’ specimens were collected from umbilical cord and the serum was removed from the clot as soon as possible. We stored our samples at -20 degree of Centigrade to avoid loss of bioactive IL-8, as kit protocol advised. The Bender Med Systems ELISA (enzyme-linked immunosorbent assay) kit was used for quantitative detection of human IL-8. The blood culture results were recorded in the patients’ files, used as the microbiology reference and were compared with our results. All statistics were calculated using the statistical package for the social sciences 15.0 for windows (SPSS Inc., Chicago). Continuous data were expressed in Mean ± Standard deviation and compared using the Student t-test as appropriate. A p-value of <0.05 was considered statistically significant. The study was approved by the university review board and informed consents were obtained from patients and/or their guardians.

RESULTS

Umbilical cord blood sample were obtained just at birth from 105 preterm neonates. Five of these neonates were excluded due to congenital anomalies. We divided 100 preterm newborns as group 1 and 2. The first group with PROM (premature rupture of membranes) and the second group included 50 premature newborns without PROM. The mean gestational age of newborns was 32 weeks, range (28-37 weeks). The mean weight of newborns were 2320 g, range 1250-2500 g. The gender of newborns was 58 boys and 48 girls. There was no correlation between IL-8 range and newborns gender. Two children were monozygotic twins. Thirty three newborns were borne by C-section and remainder by vaginal delivery. The method of labor had independent affect on IL-8 levels p = 0.36. The level of IL-8 in cord blood was not affected by gestational age p = 0.76. We have not any positive culture and it was not different in two groups. Thirty women were nulipar. There was significant difference in IL-8 level in these two groups p = 0.03. The mean level of IL-8 in study group was 128.12 pg mL⁻¹ and mean level of IL-8 in control group was 39.02 pg mL⁻¹ (Fig. 1). We showed significantly elevated values (p<0.0003) in cases with PROM (medians of 67.5 pg mL⁻¹ compared to a median of 29.5 pg mL⁻¹ without PROM).

DISCUSSION

Neonatal sepsis occurs in 1 to 21 infants per 1000 live birth. It remains a diagnostic and treatment challenge for modern neonatal care providers, with mortality rates as high as 30 to 69% of affected infants. Developing countries have both the highest incidence and the mortality rates (Stoll, 2001). Low-Birth-Weight (LBW) infants are at the highest risk for both early- and late-onset neonatal sepsis (Bromberger et al., 2000).

Isolation of microorganism(s) from one or more blood cultures is the gold standard to establish a definitive diagnosis of neonatal sepsis (Weinberg and Powell, 2001; Poureyrous, 1993). The sole use of blood culture for diagnosis of neonatal infection has a number of limitations. It may take 24 to 72 h to obtain culture results (Puts, 2000). The sensitivity of blood cultures may be impaired by exposure to intrapartum antibiotics, which are administered to 15 to 40% of mothers in labor (Bromberger, 2000; Weinberg and Powell, 2001). Intrapartum antibiotic exposure can result in a partially treated infant, delaying the onset of clinical signs and symptoms of infection and further complicating the expedient definitive diagnosis of early-onset sepsis in the infant.
The most common microbial isolates form the amniotic cavity from women with preterm labor and intact membranes are Ureaplasma urealyticum, *Fusobacterium* species and *Mycoplasma hominis* (Romero and Mazor, 1988; Pourcyrous, 1993; Prats, 2000; Frnaz, 1999).

According to a previous study carried out in Al-Zahra Obstetrics and Gynecology Hospital regarding septicemia in neonates born from mothers with PROM, 95.3% of blood culture results were negative and among the positive results *Staphylococcus aureus* and *Staphylococcus epidermidis* were the only isolated organisms.

The Medias used for isolating bacteria from blood samples in the hospital’s microbiology lab were blood agar and chocolate agar which are appropriate for isolation of streptococcus species and gram-negativ bacteria such as *Escherichia coli*. As mentioned earlier, some studies reported the pathogenic role of other species involved in intra-amniotic infection which are not growing on regular medias; this can be a factor to miss positive cultures.

Why did we chose IL-8 as a marker? Acute inflammatory illnesses including the sepsis syndrome often include a component of coagulation. A human whole blood culture system was developed so that the relationship between coagulation activation and cytokine responses in the presence or absence of lipopolysaccharides (LPS) could be evaluated. In the absence of LPS stimulation, coagulation activation resulted in a novel pattern of cytokine production. During a 4 h culture of coagulating blood, significant production of IL-8 was observed, whereas other proinflammatory cytokines including IL-1 beta, IL-6 and TNF-α were undetectable (Johnson et al., 1996; Harbarth et al., 2001). At April 2000 in the People's Republic of China, Zhang et al. (2000) studied the changes in IL-6, IL-8 and TNF-α levels in patients with PROM and found that the maternal serum IL-6, IL-8 and amniotic fluid IL-6, IL-8 and TNF-α levels were higher than those of controls. According to their study, determining cytokine levels (IL-6, IL-8 and TNF-α) is a valuable clinical method for identification of chorioamnionitis in patients with PROM.

In another study carried out in Poland at August 2000, the researchers evaluated the maternal serum cytokines levels in pregnancies complicated by PROM. Compared to healthy pregnant women, the patients with PROM had significantly higher serum levels of IL-1 β (0.76 pg mL⁻¹ vs. 0.41 pg mL⁻¹, p<0.022), TNF-α (1332.46 pg mL⁻¹ vs. 58.01 pg mL⁻¹, p<0.0001) and IL-8 (15.79 pg mL⁻¹ vs. 0 pg mL⁻¹, p<0.0001), with no considerable differences in I-6 and IL-4 levels. Differences in maternal serum levels of cytokines between patients with PROM and healthy pregnant women suggest that etiologic factors and/or consequences of PROM result in changes in immunological system (Czajkowska et al., 2000). This study showed that increased cord blood level of IL-8 can predict neonatal sepsis and can help us in monitoring of high-risk neonates and early reorganization of neonatal sepsis.

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**REFERENCES**


